

Memorandum

To: Doug Wachob, The Nature Conservancy
cc: Dave Chambers, Center for Science in Public Participation;
Carol Ann Woody, Fisheries Research and Consulting
From: Ann Maest, Joshua Lipton, and Cameron Wobus, Stratus Consulting Inc.
Date: 1/26/2010
Subject: Pebble Project Copper Bioavailability Proposal

Stratus Consulting is pleased to present a proposal to The Nature Conservancy (TNC) to develop an evaluation of the potential bioavailability of copper to salmonids at the Pebble Project area in Alaska. This memorandum provides an introduction to the issue, a discussion of the study approach, and a proposed budget. The bioavailability study would require the collection of original data in the laboratory and potentially in the field, would interface with ongoing water quality and fish population work, and would require the cooperation of the water quality sampling team as they collect their next rounds of samples at the site. We propose two phases of the study: a laboratory-based analytical and copper titration study and an associated field-based toxicity study for calibration of the laboratory results.

1. Proposed Work

1.1 Introduction and Issue Overview

The objective of the proposed work is to simulate and understand the bioavailability of copper to salmonids under baseline and operational conditions at the proposed Pebble Project. The Pebble Project is a proposed copper and gold mine in southwestern Alaska, and releases of copper from mine operations are potentially harmful to salmonids.¹ Understanding the potential bioavailability and toxicity of copper to salmonids in site waters in the vicinity of the Pebble Project is a critical element of any reasoned approach to evaluating the potential environmental consequences of mine development.

The toxicity of copper in natural waters varies as a function of its chemical makeup. Although a number of dissolved constituents contribute to the relative toxicity of copper in freshwater, primary controlling variables include calcium, pH, and dissolved organic matter (DOM). These substances influence copper toxicity through a series of geochemical processes related to

1. Other metals (e.g., cadmium, zinc, lead) and metalloids (e.g., arsenic) also could be released into the environment and are potentially harmful to salmonids. We have focused on copper because of the likely magnitude of discharges from the copper/gold mine coupled with the toxicity of this metal to salmonids and invertebrates.

competition for uptake sites on fish gills, as well as the formation of complexes that are less toxic than uncomplexed (“free”) metal. Specifically, copper toxicity tends to be reduced in harder waters with higher calcium concentrations, as well as in waters with higher amounts of DOM (the relationship with pH is somewhat more complex). Calcium hardness derives from the weathering of rock-forming minerals (primarily carbonates and feldspars). DOM derives largely from the decomposition of terrestrial and aquatic vegetation and biota.

The U.S. Environmental Protection Agency (EPA) has recently accepted the use of a model known as the Biotic Ligand Model (BLM) as the approach to calculating water quality criteria for copper to aquatic biota (Di Toro et al., 2001; U.S. EPA, 2003). All models have inherent uncertainty, but research comparing the results of the BLM with actual fish toxicity data has shown that the model often underpredicts the toxicity of copper to aquatic biota in the presence of DOM (De Schampelaere et al., 2004; Welsh et al., 2008). One potential reason for this underprediction involves the simplified treatment of DOM-Cu complexation in the BLM (e.g., Lipton et al., 2006; Welsh et al., 2008). Indeed, research has shown that the site-specific nature of DOM (including factors such as the nature, strength, and capacity of binding sites for copper and other metals and complexing agents) can influence the bioavailability and toxicity of copper (see, for example, MacRae et al., 1999; Marr et al., 1999; Welsh et al., 2008). The combination of DOM and calcium (higher hardness) leads to competition between calcium and copper for DOM binding sites, and possibly to changes in the structure of DOM (Breault et al., 1996). In addition to constituents that are dissolved in water, stream sediment can affect the bioavailability of copper. Copper can bind with organic matter or iron hydroxide coatings on particulates in streams and further decrease the amount of copper directly available to fish.

We have proposed a mixture of desktop, laboratory, and field activities to address overall project objectives. Desktop analyses would be performed to develop an understanding of the nature of potential site-specific influences on copper toxicity. Controlled laboratory studies would be performed to assess the copper-binding characteristics of natural waters potentially influenced by the Pebble Project. Finally, we have also proposed conducting an on-site streamside toxicity test. This testing would be conducted in the field using site water and local salmonids and would provide a direct measurement of the toxicity of copper in site waters and would be used to calibrate modeling results. The studies are linked but separable (and are costed out separately in the budget). In combination, the different study phases would address copper bioavailability/toxicity directly while also allowing extrapolation of results to a broader range of field conditions, thus enabling us to consider how copper toxicity might vary spatially and temporally.

Overall, the aims of the proposed work are four-fold:

- ▶ Characterize the DOM present in the three main drainages at the site (North Fork Koktuli, South Fork Koktuli, and Upper Talarik) to better understand its ability to complex or bind copper
- ▶ Simulate the addition of dissolved copper to the streams under operational conditions to estimate how much of the copper will be complexed with the natural DOM and particulate matter, how strong complexes are likely to be, and the stability of such complexes under natural conditions
- ▶ Estimate, using the BLM and scientifically supportable modifications of the BLM, how the DOM-bound copper will affect the toxicity of copper to salmonids that spawn, rear, and reside in the main drainages at the site
- ▶ Calibrate the modeling results with an on-site toxicity test to evaluate the toxicity of copper to local salmon in natural site waters.

The results of these analyses will be used in concert to develop a robust and defensible understanding of the likely bioavailability of copper to resident and migratory salmonids.

1.2 Field and Laboratory Approach

The proposed work contains desktop, field, and laboratory elements.

1.2.1 Desktop efforts, identification of sample locations, and collection of water samples

The first step will involve the review of existing Pebble Limited Partnership (PLP) and TNC water quality data to examine the distribution, temporal variability, and concentration ranges of DOC and suspended matter. DOC was not measured at every location during every sampling event, but approximately 10% or more of the water samples collected were analyzed for DOC by PLP. In recent sampling efforts by TNC (Zamzow, 2009) DOC was measured in every sample. Suspended matter has been measured routinely at the site as total suspended solids (TSS). Stratus Consulting will work in collaboration with TNC and the Center for Science in Public Participation (CSP2) to complete a database of PLP and TNC water quality, habitat, and salmonid population data. Graphs and maps of DOC and TSS values will be created to evaluate the geographic and concentration distribution over time in the three watersheds. The location of wetlands, salmon rearing and spawning areas, and potential mine facility location areas will also be mapped.

The information from the desktop studies will be used to establish, in collaboration with TNC and others in the working group, sampling locations for the collection of water for the laboratory bioavailability studies. A short sampling and analysis plan for the proposed work will be created before the first sample collection. Sample locations will be selected in each of the three major watersheds at the site (North Fork Koktuli, South Fork Koktuli, Upper Talarik). Ideally, three locations in each watershed will be identified, and the locations will correspond with existing PLP and/or TNC water quality and habitat locations and be coordinated with upcoming 2010 water quality sampling. Quality assurance samples (field duplicates) will be collected at one location in each watershed. The amount of sample needed for the laboratory studies will be determined in advance of the field sampling effort, but the volume required at each of the nine locations will be approximately 50 L, collected in darkened amber glass or Teflon bottles. Stream bottom or bank sediment will also be collected at each location in 5-L polyethylene bottles. Samples for laboratory bioavailability studies will be collected during the ice breakup and summer sampling efforts and will be shipped by overnight service to the University of Colorado laboratory in Boulder, Colorado.

1.2.2 Laboratory copper binding studies

The laboratory copper binding studies include analysis of the DOM and the performance of copper titration studies. Several analyses will be conducted at the University of Colorado laboratories upon receipt of the water samples. The analyses will help us to estimate the type and strength of binding sites available on the DOM. The measurements will include:

- ▶ Dissolved and total organic carbon (DOC and TOC), measured by a total organic carbon analyzer (Oceanography International, Model 400).
- ▶ Specific ultraviolet absorbance (SUVA), a ratio of the absorbance of light at 254 nm wavelength divided by the DOC concentration, with the absorbance measured using a ultraviolet (UV)/visible spectrophotometer (Hewlett Packard model 8453). $SUVA_{254}$ provides an indication of the aromaticity of the DOC, and aromaticity usually correlates with stronger metal binding.
- ▶ The proportion of humic and fulvic acid in each sample. Humic acids have different copper binding affinity than fulvic acids, and the relative amounts are used as inputs to the BLM.
- ▶ Elemental nitrogen analysis, as measured using a Shimadzu TOC-V_{CPH} carbon analyzer with TNM-1 total nitrogen measuring unit. Metals form stronger bonds with ligands (negatively charged pieces of DOC that bond with metals) containing nitrogen.

- Major, minor, and trace element chemistry. Ideally, this information will be available from the water quality sampling effort. The background water quality is an important component of the water quality modeling that will be used to estimate the amount of “free” (unbound) and complexed copper during the titration studies.

DOM would be isolated from the Pebble site waters and re-dissolved in waters of defined and buffered chemistry that would match the inorganic chemistry of the site waters. Titration studies would be conducted on whole water samples from the site and on laboratory waters with isolated site organic matter. The studies using laboratory waters would not contain particulates or colloids from the stream, and the results could be used directly in the BLM. The titration study will employ a copper ion-specific electrode, which measures only free copper. Copper bound to DOC, inorganic ligands, or sediment will not register on the electrode. If the amount of total copper added to the samples by titration is known, the amount and proportion of free copper can be estimated using the free copper measured by the electrode. The free copper is the form of the metal that is most toxic to salmonids.

A number of factors can influence the amount of free copper in a solution, including the presence and strength of UV light, the presence and concentration of other complexing agents or ligands, such as bicarbonate and sulfate, and the extent of association of copper with sediment. UV light can break down copper-DOC complexes and form more free copper. In Alaska, which has long days in the summer (during salmon spawning and rearing seasons), the incident UV radiation could be an important factor in the speciation of copper and the stability of organic complexes. The effect of incident UV radiation will be tested by conducting titration studies on samples kept in the dark and on others exposed to light using a solar radiation simulator.

Sulfate is a common anion associated with the formation of acid drainage. At sites such as the Pebble Mine, which have sulfide ore bodies in rocks with low buffering capacity, the formation of acid drainage and sulfate in mine drainage water is expected under operational conditions. The importance of increasing sulfate concentrations on the speciation of copper will be tested by adding sodium sulfate to a subset of the titration samples. The effects of calcium hardness will be investigated by adding calcium nitrate to a subset of the titration samples.

The rivers in the Pebble Project area are cut into glacial sand and gravel outwash plains. The size of the substrate is important for salmonid spawning (creation of redds) and promotes the availability of oxygen to the eggs. The substrate may also provide binding sites for copper released from mining operations. A subset of the titration studies will test the importance of the association of copper with the sediment by adding stream sediment to the titration samples.

Finally, the stability of the copper-DOC complexes will be tested by allowing titrated copper solutions to equilibrate for different periods of time. Previous work has shown that although copper can form strong bonds with DOC, the complex may break down over time, eventually

increasing the toxicity of copper to aquatic life over the long term. In addition, enough time needs to be allowed for the copper-DOC complex to form initially (Welsh et al., 2008).

1.2.3 Evaluation of toxicity to salmonids

Desktop evaluation

The results of the analytical and laboratory studies will be used as inputs to the BLM. The BLM will be run “as is,” using the most recent version of the model, and again making adjustments to correct for known shortcomings of the model (Welsh et al., 2008). We will run a separate geochemical model using the complete chemical analysis of the samples to estimate the amount of free copper in solution, and use that value in the BLM. The BLM will compute toxicity in terms of an LC50 concentration (the concentration of copper that will kill 50% of the salmon population). We will use different inputs and adjustments to the BLM to calculate different LC50 values and provide a discussion of the validity of each toxicity value.

Modeling work will be undertaken with the field calibration bioassay (below) to develop a calibrated toxicity model that can be contrasted against the published version of the BLM.

Field calibration bioassay

Calibration of the BLM requires an estimate of the lethal concentration of copper to 50% of a given species/strain of fish (LC50) over a given period of time. The empirical database that the BLM draws on to estimate metal toxicity to fish contains only laboratory-derived LC50s for fathead minnows (warm water fish surrogate species) and rainbow trout (coldwater fish surrogate species) in addition to data for various invertebrate species. The BLM does not contain baseline LC50 data for any salmon species. Furthermore, LC50 values can vary widely for different genetic strains of the same fish species (e.g., Meyer et al., 2007). However, if the LC50 value for a particular strain of fish is determined experimentally, this value can be used in the BLM to predict the toxicity of various water quality scenarios (i.e., various pH levels or DOC concentrations) to this specific strain. Therefore, we propose to conduct an on-site toxicity test with a native salmon species/strain (e.g., Chinook or Coho salmon) using water from their native streams under natural field conditions to determine copper LC50s that will be used to calibrate the BLM.

The field calibration will entail performing a streamside flow-through bioassay along one of the site streams (to be determined following the desktop review of existing data). The streamside bioassay would entail exposing juvenile salmon (obtained either from field collections or from Alaska Department of Fish and Game or U.S. Fish and Wildlife Service hatchery stocks) to copper added to site water. The streamside bioassay will be conducted using methods approved by EPA for standardized testing in the laboratory, modified to facilitate testing in the field.

Proper testing, waste disposal and animal collection/testing permits will be obtained, and any hatchery fish used will be certified pathogen free prior to transportation to the field site.

This field calibration will bring the laboratory to the stream, exposing native fish to serial dilutions (e.g., 35 or 50% dilutions) of dissolved copper concentrations to determine a site-specific copper LC50 to salmon. Standard laboratory protocols will be employed in which test water with varying copper concentrations (e.g., 0–250 µg/L, or parts per billion) is delivered to aquaria containing multiple (~10) larval/juvenile salmon under flow-through conditions for 96 hours. Each treatment would be replicated to ensure statistical power. Early life stage salmon will be tested, as this is the most sensitive life stage to copper. A flow-through design will be used to ensure that other water quality parameters such as pH, dissolved oxygen, temperature, DOC, and ammonia do not vary from ambient water quality conditions in the stream. During testing, water quality and fish survival will be monitored regularly, and water samples will be collected for analysis of specific parameters such as copper and DOC. The estimated volume of copper-containing effluent that will be generated through this testing will be about 1,800 L (475 gallons), and the copper concentration in this mixed effluent will be about 100 µg/L. All copper-laden effluent will be treated onsite using proven adsorption/filtration technologies such as ion exchange resins, polymeric adsorbents, and (or) chemical precipitation. Prior to discharge, chemical analysis will be conducted on treated effluent to ensure copper concentrations are at or below ambient stream copper concentrations.

The results of the field bioassays will be used in conjunction with the laboratory and desktop efforts described above to develop a calibrated understanding of the potential toxicity of copper to resident strains of salmon in natural site waters. The site- and species-specific data generated during this experiment will be invaluable in terms of modeling the effects of deleterious water quality to salmon populations under operational conditions and addressing issues regarding the extrapolation of laboratory-based results to field conditions.

2. Proposed Timeline, Deliverables, and Budget

All of the analyses described in Section 1 will be conducted during the spring/summer of 2010, with a target completion date of late 2010.

Our proposed budget is outlined in Table 1. Four tasks are contained in the budget. The first three cover the laboratory bioavailability studies: reviewing available data and preparing a sampling and analysis plan for the laboratory studies; conducting the laboratory analyses and titration studies; and preparing a summary report. Costing for all work related to the streamside toxicity studies is contained in Task 4, including preparing a sampling and analysis plan, preparation for the field studies, time in and travel to the field, travel costs, and preparation of a summary report.

The project would be managed by Ann Maest at Stratus Consulting. Dr. Joseph Ryan at the University of Colorado in Boulder, Colorado, would contribute to laboratory analytical work on the field samples and conduct the titration studies. Modeling would be conducted by Stratus Consulting staff, including Dr. Kaylene Ritter and Dr. Jeff Morris, and Dr. Ryan at the University of Colorado. Dr. Morris would be responsible for performing the streamside bioassays, with assistance from the TNC Aquatic Resources Group. Dr. Joshua Lipton, of Stratus Consulting, will provide technical and strategic advice throughout the project and will serve as officer-in-charge and technical reviewer. Michael Carney, of Stratus Consulting, will write the sampling and analysis plan and assist with laboratory analysis, if needed. David Cacela, statistician, will conduct a statistical analysis of the data from the titration studies and the stream bioassays, and will assist in the review of available data from the PLP website. Mr. Thomas Hodgson will prepare maps of the study area for the sampling and analysis plan and the final report and will assist in the organization of PLP and TNC data into the database. We have included time for administrative assistance (Laura Cross), contracts (Ray Lisk), report formatting (Erin Miles and Diane Callow), and library cataloguing (Sue Visser).

We anticipate that field samples for the laboratory studies would be collected by TNC Aquatic Resources Group Water Quality Team, and the team will also serve as collaborators and reviewers of the project approach and sampling and analysis plan.

The deliverables for the laboratory study and the field toxicity study will include short sampling and analysis plans and an overall summary report. The report will present the analytical and titration study results and the results of the streamside toxicity tests and will draw conclusions about the potential bioavailability of copper to salmonids under operational conditions in the Pebble Project area.

References

- Breault, R.F., J.A. Colman, G.R. Aiken, and D. McKnight. 1996. Copper speciation and Binding by Organic Matter in Copper-Contaminated Streamwater. *Environ. Sci. Technol.* 30(12):3477–3486.
- De Schamphelaere, K.A.C., F.M. Vasconcelos, F.M.G. Tack, H.E. Allen, and C.R. Janssen. 2004. Effect of dissolved organic matter source on acute copper toxicity to *Daphnia magna*. *Environ. Toxicol. Chem.* 23:1248–1255.
- Di Toro, D.M., H.E. Allen, H.L. Bergman, J.S. Meyer, P.R. Paquin, and R.C. Santore. 2001. Biotic ligand model of the acute toxicity of metals. Technical basis. *Environ. Toxicol. Chem.* 20:2383–2396.

Lipton, J., K. Ritter, A. Maest, and C. Mebane. 2006. Evaluating Acute Cu Toxicity to Rainbow Trout: Cu-DOC Complexation and the BLM. Presented at SETAC Europe 16th Annual Meeting, The Hague, The Netherlands, May 7–11.

MacRae, R.K., A.S. Maest, and J.S. Meyer. 1999. Selection of an organic acid analogue of dissolved organic matter for use in toxicity testing. *Can. J. Fish. Aquat. Sci.* 56(8):1484–1493.

Marr, J.C.A., J. Lipton, D. Cacela, J.A. Hansen, J.S. Meyer, and H.L. Bergman. 1999. Bioavailability and acute toxicity of copper to rainbow trout (*Oncorhynchus mykiss*) in the presence of organic acids simulating natural dissolved organic carbon. *Can. J. Fish. Aquat. Sci.* 56(8):1471–1483.

Meyer, J.S., S.J. Clearwater, T.A. Doser, M.J. Rogaczewski, and J.A. Hansen. 2007. Effects of water quality on acute toxicity of waterborne metals. In *Effects of Water Chemistry on Bioavailability and Toxicity of Waterborne Cadmium, Copper, Nickel, Lead, and Zinc to Freshwater Organisms*. SETAC Press, Pensacola, FL. pp. 41–96.

U.S. EPA. 2003. *The Biotic Ligand Model: Technical Support Document for Its Application to the Evaluation of Water Quality Criteria for Copper*. EPA #822-R-03027. November. Available: <http://www.epa.gov/waterscience/criteria/copper/2007/blm-tsd.pdf>. Accessed January 2010.

Welsh, P.G., J. Lipton, C.A. Mebane, and J.C.A. Marr. 2008. Influence of flow-through and renewal exposures on the toxicity of copper to rainbow trout. *Ecotoxicology and Environmental Safety* 69:199–208.

Zamzow, K. 2009. Pebble and Peripheral Watersheds Water Quality Study. TNC Aquatic Resources Group Water Quality Team. 2009 Field Sampling Plan. Prepared for The Nature Conservancy, Anchorage, Alaska by Center for Science in Public Participation.